Salmonella genes involved in attachment on carcass surface and pork meat contamination

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Salmonella genes involved in attachment on carcass surface and pork meat contamination

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BACKGROUND

Human infections caused by *Salmonella* from contaminated food is a common problem. Consequently, there is a need for optimizing food production environment in order to reduce any threat to human health caused by the food chain.

AIM

Establish a new approach to identify and use genes of *Salmonella* involved in the attachment on carcass surfaces and in pork chain contamination. With the design of a new meat surface model, we will analyze a number of meat and non-meat isolates for their ability to express the attachment genes. Subsequently, we will look at the time-course of the expression profile of attachment, virulence and lag-phase genes to see if specific probes can be designed to be used for ”early-warning” assays.
This may give us an idea of what kind of food products and production environments that facilitate expression of lag-phase genes.

STRATEGY

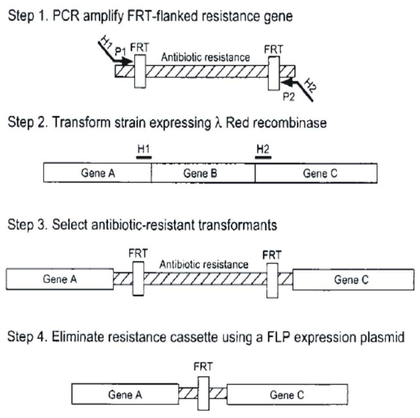
- Identify genes involved in attachment to meat
- Study the influence of the “history” of *Salmonella* on attachment potential
- Measure the expression of selected genes over time for *Salmonella* on a meat surface

METHODS

1. Identify genes involved in attachment to meat

- Genes are selected from literature studies and expression data from gel cassette system (KU-Life)
- Selected genes:
  - flagellar genes (*fli*, *flj*, *flh*, *flg*, *mot*)
  - fimbriae and curli genes (*agf*, *fim*, *pef*, *lpf*, *std*)
  - genes related to extracellular matrix (*prg*, *bap*, *mis*, *shd*)
  - genes involved in cellulose biosynthesis (*yhj*, *bcs*)

- Knock-out mutants are constructed using lambda red system (Datsenko & Wanner, 2000) and P22 transduction
- Strain: *S.* Typhimurium 4/74



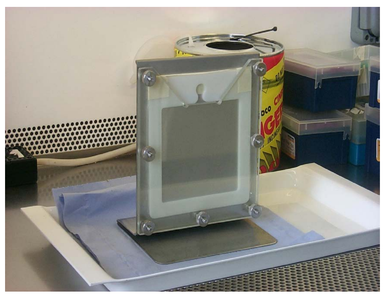
Datsenko & Wanner, 2000

- The ability to attach to meat for mutant cells and wild type cells is compared

2. Attachment studies – “history of cells”

- Influence of physiological state of *Salmonella* on adhesion is studied:
  - Liquid state – broth
  - Immobilized state – gel cassette

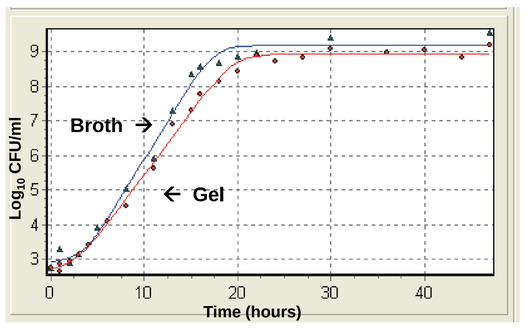
- Gel cassette system developed by IFR (Brocklehurst et al., 1995)



Gel cassette ready for inoculation medium (IFR)

- Growth experiments in broth + gel:

- Temperature: 25 °C
- Medium (gel): LB + 22% pluronic
- Medium (broth): LB

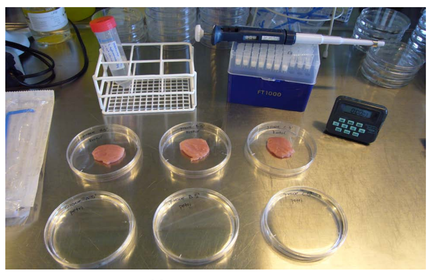


Growth curves for *S.* Typhimurium 4/74

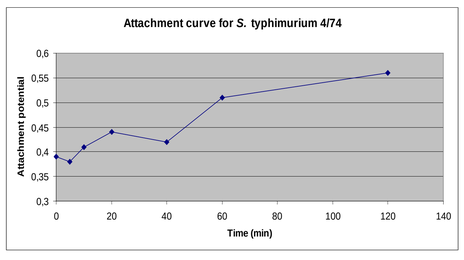
- Directly from broth and gel cassette cells are applied to a meat model at the same inoculation level (~ 10^8 CFU/ml)

3. Attachment studies – pork meat surface model

Salmonella cells from broth and gel are diluted ten times in sterilized maximum recovery diluent (MRD) and inoculated onto pieces (9.6 cm^2) of pork fillets (meat model)



Meat model



Initial test study of attachment potential of *Salmonella* to pork meat

Inoculation level (CFU/ml)	Strain	Adhesion time (min)	Temperature	Treatment	Attachment potential
~ 10^7 500 µl spread on meat surface	*S.* Typhimurium 4/74: wt vs. mutant gel vs. broth	0, 5, 10, 30, 60, 120	Room temp.	Loosly attached: wash in 40 ml MRD for 1 min Strongly attached: stomach in 40 ml MRD for 1 min	Calculated as: Strongly Strongly+Loosly

4. Time-course of gene expression on meat

- Isolate RNA from cells strongly attached to meat over time:

  - Qiagen RNeasy mini kit using combined enzymatic lysis and Proteinase K digestion
  - Quality check using Bioanalyzer
  - Conversion of total RNA into cDNA
  - Preparing for expression analysis either by whole genome microarray technology or by quantitative RT-PCR
  - Genes of interest: attachment, virulence and lag-phase

- Optimization needed:
  - Minimize background flora?
  - Increase amount of cells extracted from the meat surface?

Microarray lab at National Food Institute, DTU

REFERENCES

Brocklehurst TF, et al. (1995). JFM 27:45-60.
Datsenko KA & Wanner BL (2000). PNAS 97:6640-6645.